

Laboratory testing of human suspected cases of novel coronavirus (nCoV) infection

Interim guidance

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1. Introduction

The purpose of this document is to provide interim guidance to laboratories and stakeholders involved in laboratory testing of patients who meet the definition of suspected case of pneumonia associated with a novel coronavirus identified in Wuhan, China (See: Surveillance case definitions for human infection with novel coronavirus, Interim guidance v1, January 2020, WHO/2019-nCoV/Surveillance/v2020.1).

For the basis of this document various existing WHO documents have been used and adapted for its purpose, including WHO laboratory guidance for MERS-CoV (1-11). As information about the etiology, clinical manifestations and transmission of disease in the cluster of respiratory disease patients identified in Wuhan is limited, WHO continues to monitor developments and will revise these recommendations as necessary.

The etiologic agent responsible for the cluster of pneumonia cases in Wuhan has not yet been fully verified, but a novel Betacoronavirus has reportedly been cultured from at least one pneumonia patient and characterized by electron microscopy and genome sequencing and has been detected by PCR in 15 other patients (12). It is expected that full gene sequence and other information on the putative causative agent will soon be available that can inform the development of specific diagnostic tests. Until that time, the goals of diagnostic testing are to detect suspect cases early, to support disease control activities, and to work with reference laboratories that can perform pathogen discovery and additional testing to clarify the pathogenic role of the putative emergent cause of respiratory disease.

2. Suspected case definition

For case definition see the following document: WHO Surveillance case definitions for human infection with novel coronavirus.

3. Specimen collection and shipment

Rapid collection and testing of appropriate specimens from suspected cases is a priority and should be guided by a laboratory expert. As the causative agent has not been verified and the gene sequence of the putative coronavirus not yet published, multiple tests may need to be performed and sampling sufficient clinical material is recommended. Local guidelines should be followed regarding patient or guardian's informed consent for specimen collection, testing and potentially future research.

Assure SOPs are available, and the appropriate staff is trained and available for appropriate collection, specimen storage, packaging and transport. There is still limited information on the risk posed by the reported coronavirus found in Wuhan, but it would appear samples prepared for molecular testing could be handled as would samples of suspected human influenza (2, 7-9). Attempts to culture the virus require a higher level of biosecurity.

Samples to be collected (see Table 1 for details on sample collection and storage):

1. Respiratory material* (nasopharyngeal and oropharyngeal swab in ambulatory patients and sputum (if produced) and/or endotracheal aspirate in patients with more severe respiratory disease)
2. Serum for serological testing, acute sample and convalescent sample (this is additional to respiratory materials and can support the identification of the true agent, once serologic assay is available)
3. Other specimens to consider in unresolved cases: blood for culture, urine for *Legionella* and pneumococcal antigen detection

*To be modified once information is available on whether upper or lower respiratory material is the better sample for detection of the putative coronavirus.

A single negative test result, particularly if this is from an upper respiratory tract specimen, does not exclude infection. Repeat sampling and testing, lower respiratory specimen is strongly recommended in severe or progressive disease. A positive alternate pathogen does not necessarily rule out either, as little is yet known about the role of coinfections.

Reference 2, 3, 7

Diagnostic algorithm for patients that meet the suspected case definition

Until the cause of respiratory disease originating in Wuhan is confirmed and a diagnostic test available, patients that meet the suspected case definition should be screened for common causes of respiratory illness according to local guidelines (1,5,7). When results of the screening are negative, a sample should be sent to a regional, national or international reference laboratory with pathogen discovery capability (e.g., sequencing, electron microscopy, viral culture). WHO can assist Member States to identify laboratories able to provide this support. Once the causative agent of this outbreak is verified and test reagents made available, specific diagnostics directed to this agent can be added to the diagnostic algorithms.

Example of differential diagnosis for pneumonia in outbreak settings:^{*}

Viral infections such as influenza -A, -B, and -C virus, adenovirus, MERS-, SARS coronavirus, other human coronaviruses, respiratory syncytial virus, parainfluenza virus 1-4, human metapneumovirus, rhinovirus/enterovirus.

Bacterial infections such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Streptococcus pyogenes*, Legionnaires' disease/Pontiac fever (*Legionella pneumophila*, and *Legionella non-pneumophila*), anthrax, leptospirosis and atypical bacterial infection such as *mycoplasma* and *Chlamydia pneumoniae* and *psittaci*, Q-fever (*Coxiella burnetii*), mycobacterial infections and in specific patient groups opportunistic infections such as PJP and fungal infections. The possibility of exposure to non-infectious agents (e.g. toxins, radiation) should also be considered depending on the clinical syndrome.

^{*}Needs adaptation in relation to local guidelines, regional occurrence of disease, specific patient risk factors and clinical presentation)

Note: Coinfections and bacterial superinfections can occur, and non-obligate pathogens detected might not be the cause of illness.

Safety procedures during sample collection and transport

All specimens collected for laboratory investigations should be regarded as potentially infectious, and HCWs who collect, or transport clinical specimens should adhere rigorously to infection prevention and control guidelines and national or international regulations for the transport of dangerous goods (infectious substances) to minimize the possibility of exposure to pathogens (14). Though the mode of transmission of the causative agent(s) is not established, HCWs should assume the potential for respiratory spread. Implement the highest available level of achievable infection prevention and control precautions according to protocol until the mode of transmission and risk from infection is clarified (11).

Infection prevention measurements for a novel coronavirus (route of transmission unknown but suspected to be respiratory)

Ensure that Health Care workers (HCWs) who collect specimens follow the following guideline and use the adequate PPE: Infection prevention and control during health care when novel coronavirus (nCoV) infection is suspected, interim guidance, January 2020 (11) and other IPC guidance (10, 15-17).

Ensure that HCWs performing aerosol-generating procedures (i.e. aspiration or open suctioning of respiratory tract specimens, intubation, cardiopulmonary resuscitation, bronchoscopy) use additional precautions (for details see detailed guidelines mentioned above).

Respirators (NIOSH-certified N95, EU FFP2 or equivalent, or higher level of protection). When putting on a disposable particulate respirator, always check the seal/fitness. Be aware that the presence of facial hair (e.g. beard) may prevent a proper respirator fit for the wearer. In some countries, a powered air-purifying respirator (PAPR) is utilized instead of a respirator.

- Eye protection (i.e. goggles or a face shield).
- Clean, non-sterile, long-sleeved gown and gloves. Note that some procedures require sterile gloves. If gowns are not fluid resistant, a waterproof apron should be used for procedures where it is expected that high fluid volumes might penetrate the gown
- Perform procedures in an adequately ventilated room: at a minimum natural ventilation with at least 160l/s/patient air flow, or negative pressure rooms with at least 12 air changes per hour and controlled direction of air flow when using mechanical ventilation
- Limit the number of persons present in the room to the minimum required for the patient's care and support; and
- Follow WHO guidance for steps of donning and doffing PPE. Perform hand hygiene before and after contact with the patient and his or her surroundings and after PPE removal.
- Waste management and decontamination procedures: Ensure that all materials used is disposed appropriately. Disinfection of work areas and decontamination of possible spills of blood or infectious body fluids should follow validated procedures, usually with chlorine-based solutions.

Specifics for transport of samples to laboratory:

- Ensure that personnel who transport specimens are trained in safe handling practices and spill decontamination procedures.
- Follow the requirements in the national or international regulations for the transport of dangerous goods (infectious substances) as applicable (14).
- Deliver all specimens by hand whenever possible. Do not use pneumatic-tube systems to transport specimens.
- State the full name, date of birth of the suspected SARI case clearly on the accompanying request form. Notify the laboratory as soon as possible that the specimen is being transported.

Assure good communication with the laboratory and provide needed information

To assure proper and fast processing of samples and to assure adequate biosafety measures in the laboratory, communication and information sharing is essential. Be sure you have alerted the laboratory of the urgency and situation before sending the sample. Also assure that specimens are correctly labelled, and diagnostic request forms are filled out properly and clinical information is provided (see box information to be recorded)

Information to be recorded:

- Patient information – name, date of birth, sex and residential address, unique identification number, other useful information (e.g. patient hospital number, surveillance identification number, name of hospital, hospital address, room number, physicians' name and contact information, name and address for report recipient),
- Date and time of sample collection,
- Anatomical site and location of specimen collection,
- Tests requested,
- Clinical symptoms and relevant patient history (including vaccination and antimicrobial therapies received, epidemiological information, risk factors).

Table 1. Specimens to be collected from symptomatic patients and asymptomatic contacts

Guidance on specimen collection (adapted from reference 5)

Specimen type	Collection materials	Transport to laboratory	Storage till testing	Comment
Nasopharyngeal and oropharyngeal swab	Dacron or polyester flocked swabs*	4 °C	≤5 days: 4 °C >5 days: -70 °C	The nasopharyngeal and oropharyngeal swabs should be placed in the same tube to increase the viral load.
Bronchoalveolar lavage	sterile container *	4 °C	≤48 hours: 4 °C >48 hours: -70 °C	There may be some dilution of pathogen, but still a worthwhile specimen
Tracheal aspirate, nasopharyngeal aspirate or nasal wash	sterile container *	4 °C	≤48 hours: 4 °C >48 hours: -70 °C	
Sputum	sterile container	4 °C	≤48 hours: 4 °C >48 hours: -70 °C	Ensure the material is from the lower respiratory tract
Tissue from biopsy or autopsy including from lung	sterile container with saline	4 °C	≤24 hours: 4 °C >24 hours: -70 °C	
Serum (2 samples acute and convalescent possibly 2-4 weeks after acute phase)	Serum separator tubes (adults: collect 3-5 ml whole blood)	4 °C	≤5 days: 4 °C >5 days: -70 °C	Collect paired samples: • acute – first week of illness • convalescent – 2 to 3 weeks later
Whole blood	collection tube	4 °C	≤5 days: 4 °C >5 days: -70 °C	For antigen detection particularly in the first week of illness
Urine	urine collection container	4 °C	≤5 days: 4 °C >5 days: -70 °C	

*For transport of samples for viral detection, use VTM (viral transport medium) containing antifungal and antibiotic supplements. For bacterial or fungal culture, transport dry or in a very small amount of sterile water. Avoid repeated freezing and thawing of specimens.

Aside from specific collection materials indicated in the table also assure other materials and equipment are available: e.g. transport containers and specimen collection bags and packaging, coolers and cold packs or dry ice, sterile blood-drawing equipment (e.g. needles, syringes and tubes), labels and permanent markers, PPE, materials for decontamination of surfaces.

4. Effective usage of Global Laboratory Networking

Timely and accurate laboratory testing of specimens from cases under investigation is an essential part of the management of emerging infections. All countries should have access to reliable testing, either nationally or internationally, in laboratories willing to perform primary detection or confirmatory testing, and novel pathogen detection. WHO can assist Member States to access testing internationally should the need arise.

5. Testing in reference laboratories

Microscopy

Light and electron microscopy can rapidly provide the first information on the potential causative agent in clinical materials. However subsequent testing is needed to identify the pathogen.

Culture

Viral culture is often considered the “gold standard” for laboratory diagnosis of viral respiratory infections. Laboratories with the appropriate experience and containment facilities, may attempt to isolate the virus. These recommendations do not cover virus isolation procedures. Culture of virus has important biosafety implications, depending on the type of virus, its pathogenicity and mechanism of spread.

Molecular identification and characterization of a novel pathogen

A number of methods and systems for rapid and sensitive identification of the genetic sequence of novel pathogens have been developed and refined. Sharing such gene sequence information among collaborators is essential to rapidly identify the pathogen and to develop pathogen-specific diagnostics.

In addition to identifying the novel pathogen, sequence data can also provide valuable information for understanding the origin of the virus and how it is spreading. WHO has published a Draft code of conduct for the handling of Genetic Sequence Data related to outbreaks (see https://www.who.int/blueprint/what/norms-standards/GSDDraftCodeConduct_forpublicconsultation-v1.pdf?ua=1). That policy framework recommends making sequence data publicly available. Different models for sharing of pathogen sequences exist, open (e.g. GenBank, virological.org) or semi-open (e.g. GISAID) platforms. Laboratories are encouraged to share sequence data with WHO and the scientific community to assist in the rapid development and distribution of diagnostic assays in at risk countries. Leading medical journals now have regulations discouraging publication of articles on outbreak pathogens

when the authors did not expedite the release of sequence information into the public sector. WHO can assist Member States to identify laboratories able to provide support and advise them on the management of sequence data related to an outbreak.

Serological testing

Serological testing may be useful to confirm immunologic response to a pathogen from a specific viral group, e.g. coronavirus. Best results from serologic testing requires the collection of paired serum samples (in the acute and convalescent phase) from cases under investigation.

In the absence of shared sequence information from the putative pathogen from the Wuhan outbreak, laboratories may desire to use a pan-coronavirus assay for amplification followed by sequencing of the amplicon for characterization and confirmation. External confirmation should be sought from a reference laboratory that can deploy additional assays. It is important to consider that four human coronaviruses (HCoVs) are endemic globally: HCoV-229E, HCoV-NL63, HCoV-HKU1 as well as HCoV-OC43. The latter two are betacoronaviruses. Two other betacoronaviruses that cause zoonotic infection in humans are MERS-CoV, acquired by contact with dromedary camels and SARS arising from civets and cave-dwelling horseshoe bats.

Once genome sequences of the novel coronavirus have been released and specific NAAT assays developed, confirmation of cases of the novel virus infection will be based on specific detection of unique sequences of viral nucleic acid by reverse-transcriptase polymerase chain reaction (RT-PCR) with probe detection or sequencing. Alternative NAAT techniques with advantages of speed or simplicity of use may also become available.

Specifics for biosafety practices in the laboratory

Ensure that health laboratories adhere to appropriate biosafety practices. Any testing on clinical specimens from patient meeting the case definition should be performed in appropriately equipped laboratories by staff trained in the relevant technical and safety procedures. National guidelines on the laboratory biosafety should be followed in all circumstances. General information on laboratory biosafety guidelines, see the WHO Laboratory Biosafety Manual, 3rd edition (8).

It is recommended that all manipulations in laboratory settings of samples originating from suspected or confirmed cases of novel coronaviruses can be conducted according to WHO recommendations available at:

https://www.who.int/csr/disease/coronavirus_infections/Biosafety_InterimRecommendations_NovelCoronavirus2012_31Oct12.pdf?ua=1 Information on biosafety levers for SARS, a Betacoronavirus that can cause severe respiratory disease can be consulted at https://www.who.int/csr/sars/biosafety2003_04_25/en/ and other guidance.

Table 1. Tests to be performed in expert laboratories for patients meeting the case definition

Test	Type of sample	Timing
Whole genome sequencing	Lower respiratory tract - sputum - aspirate - lavage	Collect on presentation, done by an expert laboratory.
	Upper respiratory tract - naso pharyngeal and - oro pharyngeal swabs - naso pharyngeal wash/naso pharyngeal aspirate	
NAAT when it becomes available Note: In laboratories that have validated broad range coronavirus RT-PCR available, this might be considered, however at this date insufficient information is available to guarantee proper detection, thus multiple broad range coronavirus assays should be used and amplicon sequencing should always be part of the algorithm.	Lower respiratory tract - sputum - aspirate - lavage	Collect on presentation. To confirm clearance of the virus, sample collection to be repeated until the results are negative on 2 sequential samples.
Serology, broad corona virus serology on paired samples if available	Upper respiratory tract - naso pharyngeal and - oro pharyngeal swabs - naso pharyngeal wash/naso pharyngeal aspirate	Multiple broad range coronavirus assays, done by an expert laboratory.
	Serum for serological testing if NAAT is not available	Paired samples are necessary for confirmation with the initial sample collected in the first week of illness and the second ideally collected 3-4 weeks later. If only a single serum sample can be collected, this should occur at least 3-4 weeks after onset of symptoms for determination of a probable case.

Packaging and shipment to another laboratory

Transport of specimens within national borders should comply with applicable national regulations. International Transport Regulations. Novel coronavirus specimens should follow the UN Model Regulations, and any other applicable regulations depending on the mode of transport being used. More information may be found in the WHO Guidance on regulations for the Transport of Infectious Substances 2019-2020 (Applicable as from 1 January 2019) (14). A summary on transport of infectious substances can also be found in Toolbox 4 of the Managing epidemics handbook (1).

Patient specimens from suspected or confirmed cases should be transported as UN3373, “Biological Substance, Category B”, when they are transported for diagnostic or investigational purposes. Viral cultures or isolates should be transported as Category A, UN2814, “infectious substance, affecting humans”. All specimens being transported (whether UN3373 or UN2814) should have appropriate packaging, labelling and documentation, as described above.

6. Reporting of cases and test results

Laboratories should follow national reporting requirements, but in general, suspected cases should be reported to relevant public health authorities as soon as the laboratory receives a specimen, even before any testing is performed. All test results, whether positive or negative, should likewise be immediately reported to national authorities. If the infection becomes widespread, laboratories should notify public health authorities immediately of each new confirmed case or positive screening test if there will be a delay in confirmatory testing. Laboratories should also periodically report the number of negative test results to public health.

States Parties to the IHR are reminded of their obligations to share with WHO relevant public health information for events for which they notified WHO, using the decision instrument in Annex 1 of the IHR (2005) (18).

Detection of a possible human case of emerging pathogen causing severe acute respiratory disease should immediately

be notified to local, subnational and national public health authorities. This will allow these authorities to make immediate decisions about launching the investigation and the extent of response measures. Detection of such a case should be used to trigger notification of traditional and non-traditional health providers, hospitals and outpatient.

Facilities, and community leaders in the area where the case patients lived or travelled, as part of active case-finding efforts. In line with the International Health Regulations (IHR) (2005), the national health authority must notify WHO within 24 hours of all events that may constitute a public health emergency of international concern according to defined criteria. The IHR decision instrument should be used to determine whether an event is to be notified to WHO. Further guidance on the use of the IHR decision instrument, including examples of its application, is available. The national animal health authority must notify OIE of certain animal diseases detected on its territory. OIE focal points should be contacted for further details.

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